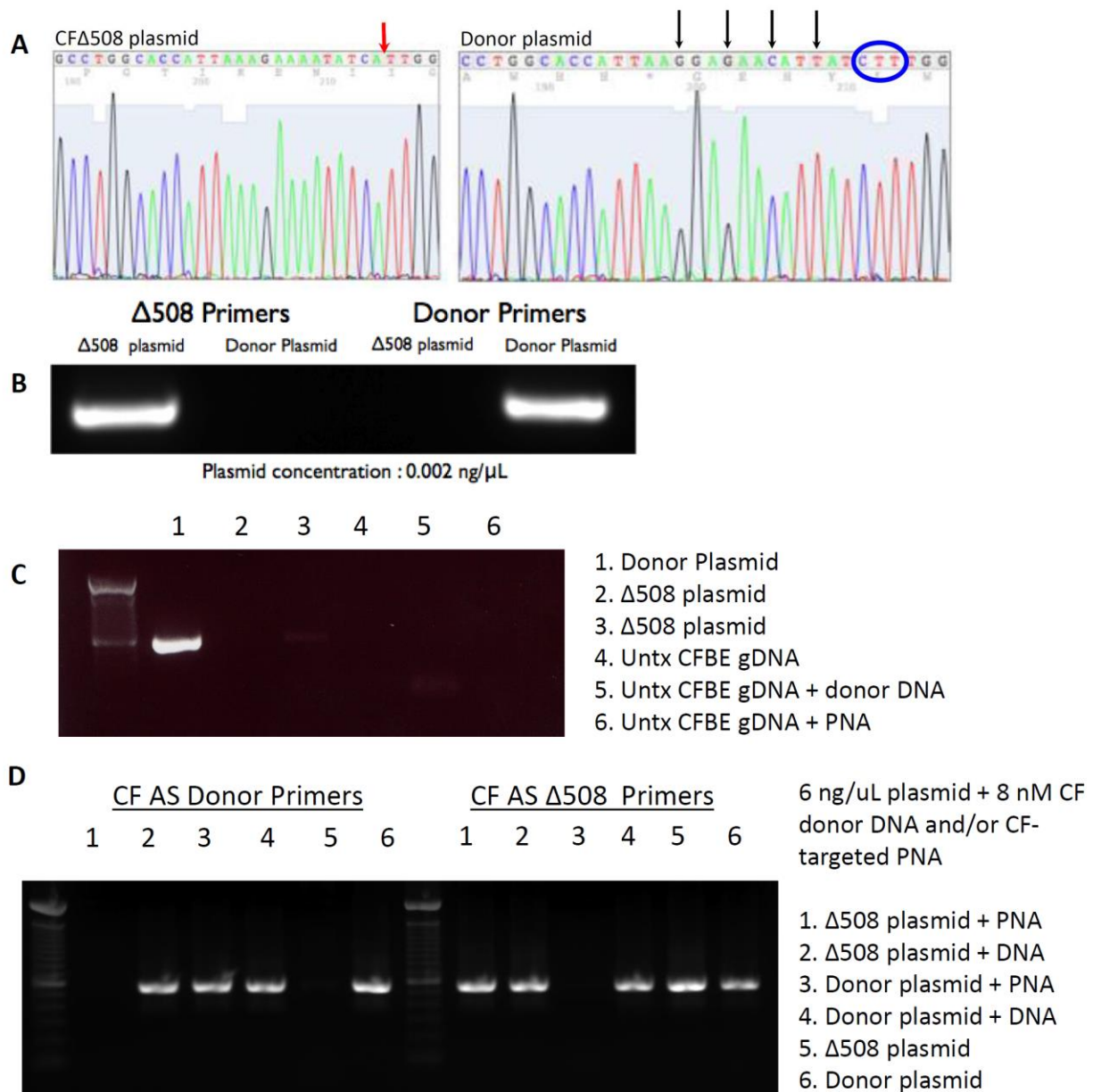
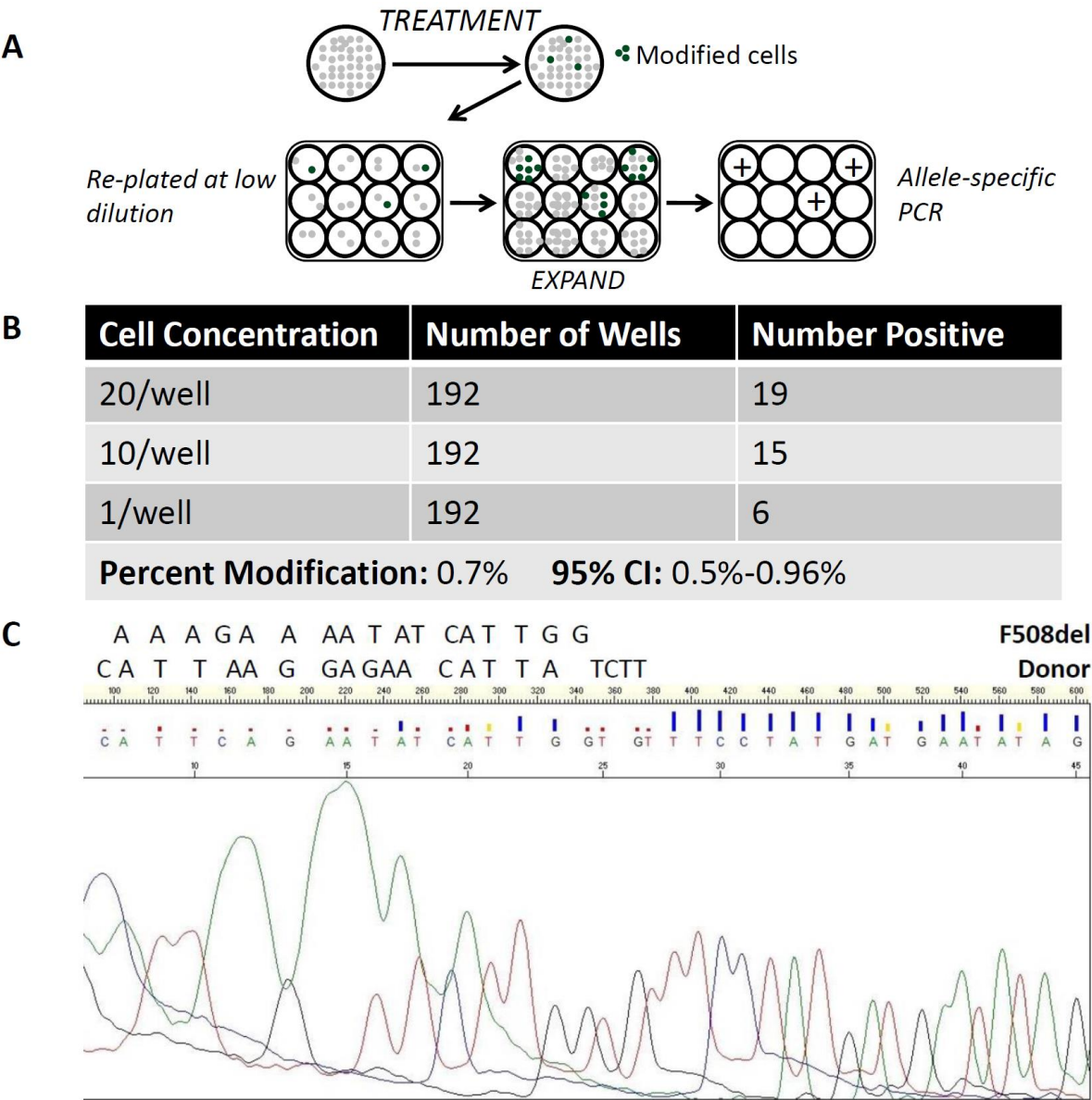


Supplementary Figure 1. PNA binding. To test the binding of each PNA to its targeted sequence in the CFTR gene we used a gel shift assay. Plasmids were created containing approximately 200 base pairs of the CFTR gene, including the PNA binding sites. CF-PNA-1 (hCF-PNA-1) binds 54 bp downstream of the F508DEL target site, CF-PNA-2 (hCF-PNA-2) binds 178 bp downstream, and CF-PNA-3 (hCF-PNA-3) binds 317 bp upstream. mCF-PNA-2 binds in murine CFTR. J represents pseudoisocytosine, a C analog for improved triplex formation at physiologic pH. To test binding, the PNAs were incubated with plasmid DNA containing the target site overnight, then the plasmid was cut with restriction enzymes flanking the binding site, and the products were analyzed by electrophoresis on an 8% non-denaturing PAGE gel, with silver stain for visualization. hCF-PNA-2 binding is shown in the main text.

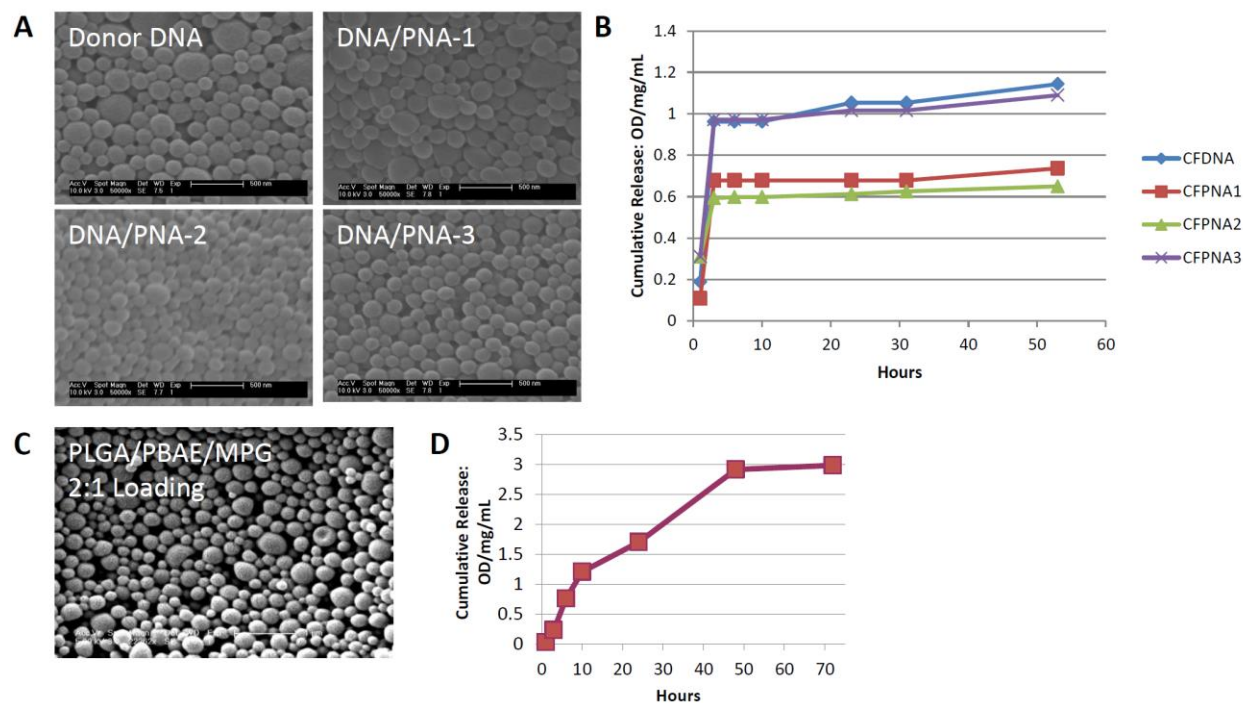


Supplementary Figure 2. AS-PCR can differentiate between corrected and F508DEL *CFTR* gene. (A) Plasmids were created containing a 712 bp segment of the *CFTR* gene containing the F508DEL site, or the sequence of the donor DNA which is designed to correct the F508DEL mutation as well as introduce some additional base pair changes to further tag the sequence for facile identification by PCR or genomic sequencing. (B) AS-PCR can differentiate between the corrected (donor) and F508DEL sequences. (C) DNA from human bronchial epithelial cells carrying the F508DEL mutation was spiked with differing concentrations of the donor DNA immediately prior to the PCR reaction. The lack of amplification in the AS-PCR assay by the donor/corrected primers, indicates that the donor DNA itself does not participate as a primer or provide a false template. (D) AS-PCR could be inhibited by high concentrations of PNA or donor DNA. Plasmid DNA with either the donor or F508DEL mutation spiked with

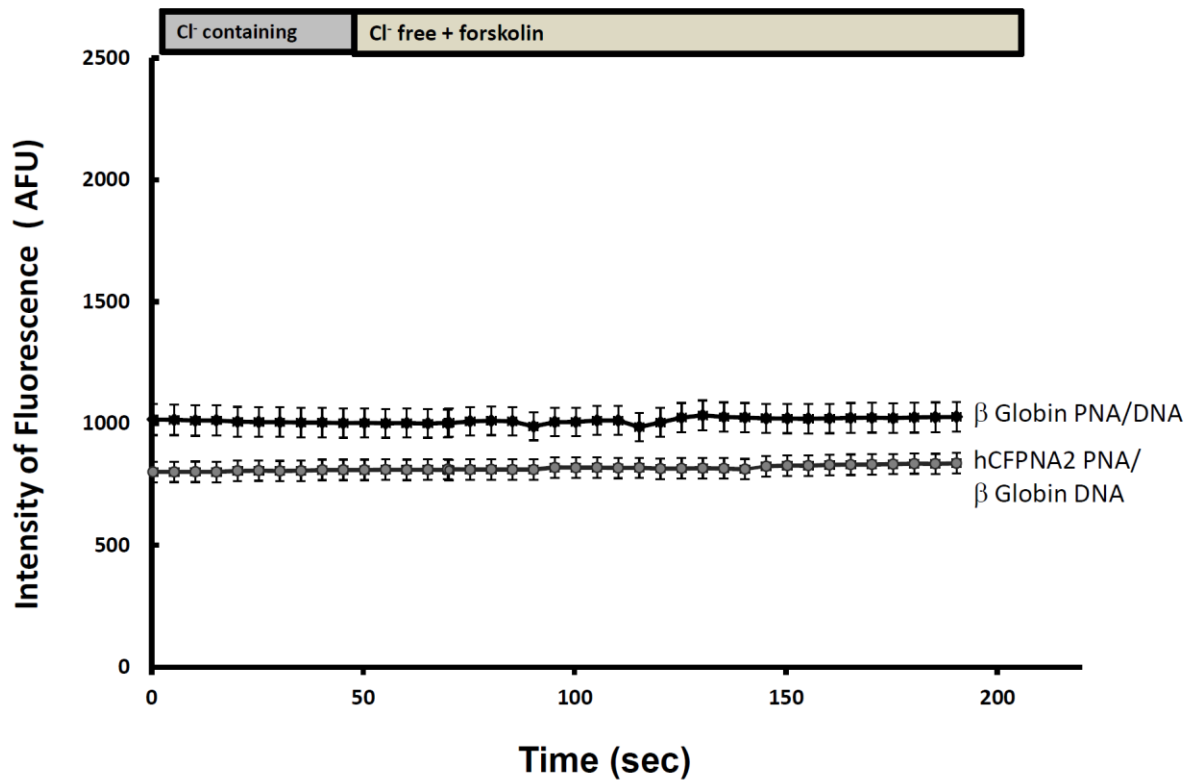
PNA and DNA at concentrations at > 10x the expected cellular level lead to inhibition of the PCR reaction.



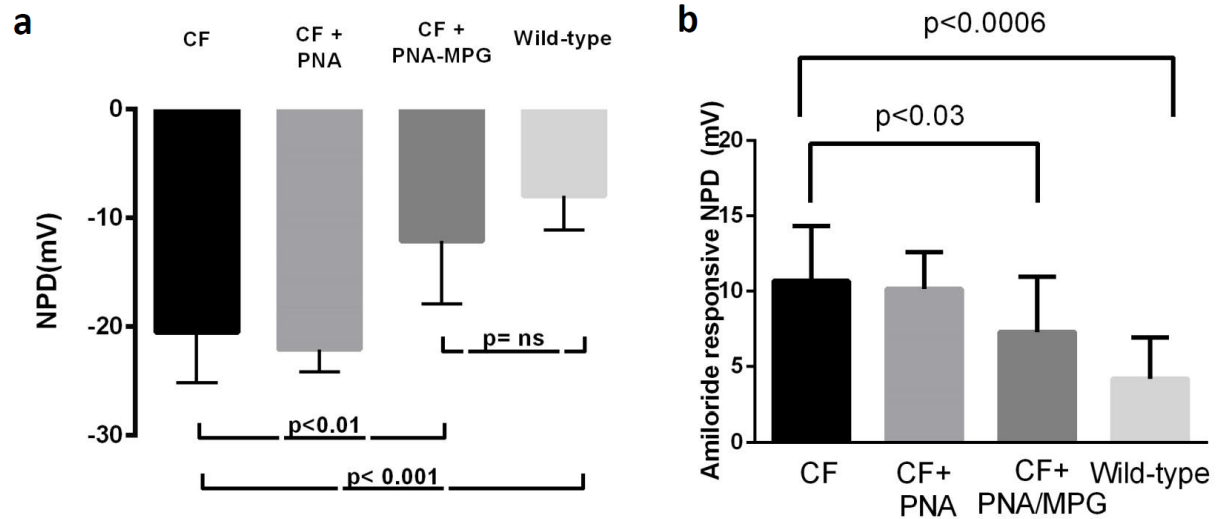
Supplementary Figure 3. Isolation of corrected cells by limiting dilution and cloning into multi-well plates. (A) Cells were plated at dilutions ranging from 100 cells/well to 1 cell/well. After expansion to produce enough cells for harvest, genomic DNA was extracted from each well, and AS-PCR used to detect presence of the corrected *CFTR* sequence. **(B)** The frequency of modification was calculated using limiting dilution analysis. **(C)** In one of the isolated clones (411), sequence modification was confirmed by regular sequencing. While Figure 2 shows sequencing of a 712 bp region of clone 411 after 35 cycles PCR amplification, here we show sequencing after amplification of a 100 bp region surrounding the modification site, after 20 cycles of PCR amplification, to avoid PCR bias. The region with our modification (3 base-pair insertion, plus 4 additional silent mutations) shows lower sequencing quality, as may be expected due to the indel, and presence of both sequences.



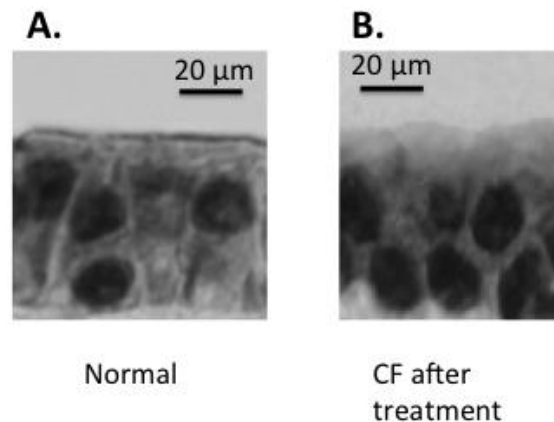
Supplementary Figure 4. Formulation of nanoparticles containing PNA/DNA targeted to *CFTR*. (A&B) PLGA nanoparticles with DNA alone or PNA:DNA loading ratio of 1:2. **(C&D)** PLGA/PBAE/MPG particles with hCFPNA2:DNA loading ratio of 2:1. **(A&C)** Nanoparticles containing the donor DNA alone or with each of the candidate PNA molecules were synthesized, and imaged by SEM. Average sizes of particles were analyzed by ImageJ: diameters were 120 +/- 40 nm for blank, 150 +/- 55 nm for CFDNA, 120 +/- 27 for CFPNA1, 140 +/- 72 for hCFPNA2, and 130 +/- 42 for hCFPNA3 particles. **(B&D)** Release of nucleic acid from nanoparticles incubated at 37°C.



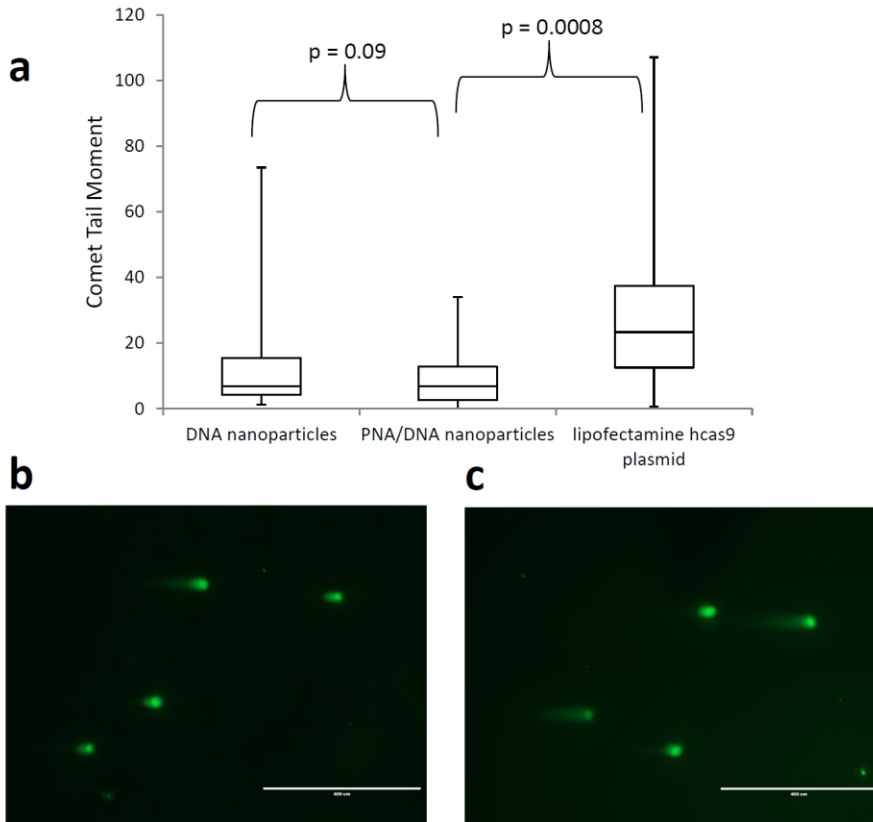
Supplementary Figure 5. Nanoparticles with PNA not targeted to CFTR show no effect on chloride efflux. Chloride efflux was measured using N-[ethoxycarbonylmethyl]- 6-methoxy-quinolinium bromide (MQAE), a fluorescent indicator dye. Cells (n=24) were treated as in Figure 4, but with PLGA/PBAE/MPG nanoparticles containing PNA/DNA targeting the human β -globin gene or with PNA targeting CFTR and DNA targeting β -globin. Error bars= standard error of the mean.



Supplementary Figure 6. Baseline NPD and amiloride response in CF, treated-CF, and wildtype mice. Mice were treated by intranasal infusion with nanoparticles. Nasal potential difference measurements were assessed prior to nanoparticle treatment, and subsequent to treatment, as shown in Figure 4. Wild-type mice ($n = 6$), untreated CF mice ($n = 18$), CF mice treated with PLGA (CF+PNA) ($n = 8$) or PLGA/PBAE/MPG nanoparticles (CF+PNA-MPG) ($n = 8$) containing PNA/DNA are shown. All error bars show SD. **(a)** Summary of baseline nasal potential difference change. NPD measurements were compared between groups using one way ANOVA with multiple comparisons. **(b)** Amiloride responsive NPD change. NPD measurements were compared between groups using one way ANOVA with multiple comparisons.



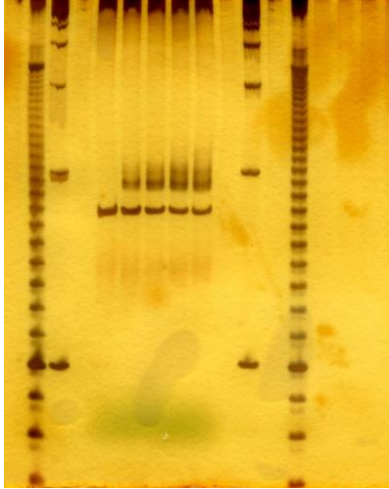
Supplementary Figure 7. Histology of nasal epithelia of treated and untreated mice, paraffin embedded and stained with H&E.



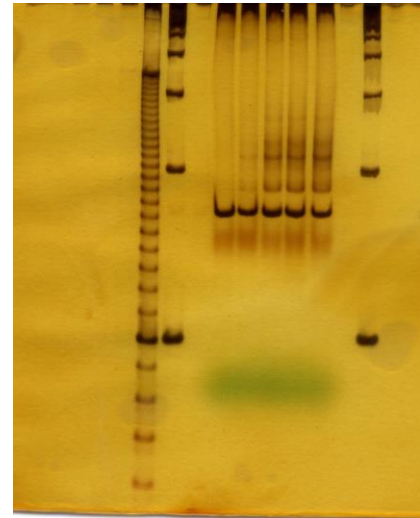
Supplementary Figure 8. Comet assay for DNA damage. CFBE cells were treated for 24 hours with 2 mg/mL DNA-containing PLGA/PBAE/MPG nanoparticles, 2 mg/mL PNA and DNA-containing PLGA/PBAE/MPG nanoparticles, or 2 ug of hCas9 plasmid (Addgene plasmid 41815). Cells were prepared per the Trevigen Comet Assay protocol, placed on slides, electrophoresed, stained with Sybr green, and visualized using an EVOS microscope. **(a)** TriTek Comet Score FreeWare was used to calculate comet tail moments, represented as box-whisker plots. Plots show the median comet tail moments (horizontal lines), min and max comet tail moments (top and bottom of vertical lines), and first to third quartile (box). P-values are for Student's ttest, two-tailed, unpaired, unequal variance. **(b)** Representative image for PNA/DNA nanoparticles. **(c)** Representative image for hcas9.

Supplementary Figure 9. UNCROPPED GEL IMAGES FOR PNA GEL SHIFTS

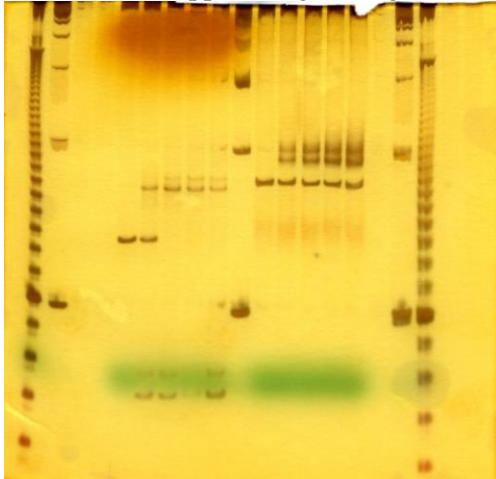
CFPNA-1: Cropped image in Supplementary Figure 1



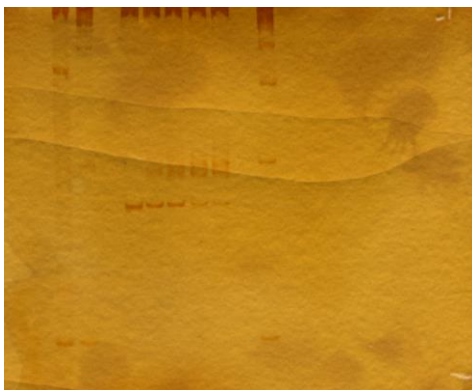
mCFPNA-2: Cropped image in
Supplementary Figure 1



CFPNA-2: Cropped image in Figure 1c



CFPNA-3: Cropped image in Supplementary Figure 1



Supplementary Figure 10.

AS-PCR on F508del and corrected plasmids: Cropped image in Supplementary Figure 2B



<u>TREATED CFBE</u> <i>proportions</i>			<u>UNTREATED CFBE</u> <i>proportions</i>		
chr15			chr15		
Total	251695		Total	530760	
0 mismatch	229686	0.91256	0 mismatch	459809	0.86632
1 mismatch	20350	0.08085	1 mismatch	61671	0.11619
2 mismatch	1507	0.00599	2 mismatch	7703	0.01451
3 mismatch	108	0.00043	3 mismatch	1214	0.00229
4 mismatch	18	0.00007	4 mismatch	234	0.00044
5 mismatch	12	0.00005	5 mismatch	80	0.00015
chr1			chr1		
Total	401797		Total	858936	
0 mismatch	357349	0.88938	0 mismatch	731641	0.8518
1 mismatch	39967	0.09947	1 mismatch	109715	0.12773
2 mismatch	3936	0.0098	2 mismatch	14826	0.01726
3 mismatch	408	0.00102	3 mismatch	2172	0.00253
4 mismatch	55	0.00014	4 mismatch	384	0.00045
5 mismatch	18	0.00004	5 mismatch	102	0.00012
chr6			chr6		
Total	461729		Total	961725	
0 mismatch	206678	0.44762	0 mismatch	412262	0.42867
1 mismatch	225397	0.48816	1 mismatch	464482	0.48297
2 mismatch	26986	0.05845	2 mismatch	73976	0.07692
3 mismatch	2290	0.00496	3 mismatch	9241	0.00961
4 mismatch	243	0.00053	4 mismatch	1352	0.00141
5 mismatch	36	0.00008	5 mismatch	203	0.00021
chr16			chr16		
Total	504467		Total	1117272	
0 mismatch	381955	0.75715	0 mismatch	825930	0.73924
1 mismatch	109055	0.21618	1 mismatch	247965	0.22194
2 mismatch	11902	0.02359	2 mismatch	36742	0.03289
3 mismatch	1236	0.00245	3 mismatch	5278	0.00472
4 mismatch	121	0.00024	4 mismatch	892	0.0008
5 mismatch	56	0.00011	5 mismatch	189	0.00017
chr3			chr3		
Total	57698		Total	134309	
0 mismatch	52424	0.90859	0 mismatch	116734	0.86915
1 mismatch	4608	0.07986	1 mismatch	14917	0.11106

2 mismatch	346	0.006	2 mismatch	1752	0.01304
3 mismatch	51	0.00088	3 mismatch	312	0.00232
4 mismatch	35	0.00061	4 mismatch	119	0.00089
5 mismatch	47	0.00081	5 mismatch	97	0.00072

chr11			chr11		
Total	343047		Total	945354	
0 mismatch	306195	0.89257	0 mismatch	805771	0.85235
1 mismatch	32269	0.09407	1 mismatch	118156	0.12499
2 mismatch	3808	0.0111	2 mismatch	17664	0.01869
3 mismatch	481	0.0014	3 mismatch	2804	0.00297
4 mismatch	77	0.00022	4 mismatch	536	0.00057
5 mismatch	22	0.00006	5 mismatch	94	0.0001

chr4			chr4		
Total	641990		Total	1395861	
0 mismatch	519442	0.80911	0 mismatch	1216147	0.87125
1 mismatch	111962	0.1744	1 mismatch	156143	0.11186
2 mismatch	9521	0.01483	2 mismatch	19992	0.01432
3 mismatch	896	0.0014	3 mismatch	2884	0.00207
4 mismatch	99	0.00015	4 mismatch	481	0.00034
5 mismatch	18	0.00003	5 mismatch	122	0.00009

chr17			chr17		
Total	94144		Total	188011	
0 mismatch	40784	0.43321	0 mismatch	77406	0.41171
1 mismatch	46505	0.49398	1 mismatch	91505	0.4867
2 mismatch	5905	0.06272	2 mismatch	15895	0.08454
3 mismatch	672	0.00714	3 mismatch	2487	0.01323
4 mismatch	73	0.00078	4 mismatch	379	0.00202
5 mismatch	66	0.0007	5 mismatch	148	0.00079

chr18			chr18		
Total	41662		Total	79529	
0 mismatch	38127	0.91515	0 mismatch	69401	0.87265
1 mismatch	3173	0.07616	1 mismatch	8755	0.11009
2 mismatch	279	0.0067	2 mismatch	1084	0.01363
3 mismatch	43	0.00103	3 mismatch	179	0.00225
4 mismatch	8	0.00019	4 mismatch	39	0.00049
5 mismatch	6	0.00014	5 mismatch	15	0.00019

chrX			chrX		
Total	111204		Total	200817	

0 mismatch	88760	0.79817	0 mismatch	148976	0.74185
1 mismatch	13159	0.11833	1 mismatch	28764	0.14323
2 mismatch	2155	0.01938	2 mismatch	5656	0.02816
3 mismatch	361	0.00325	3 mismatch	1170	0.00583
4 mismatch	94	0.00085	4 mismatch	249	0.00124
5 mismatch	71	0.00064	5 mismatch	148	0.00074

chr8			chr8		
Total	72801		Total	147441	
0 mismatch	65711	0.90261	0 mismatch	126820	0.86014
1 mismatch	6430	0.08832	1 mismatch	17720	0.12018
2 mismatch	496	0.00681	2 mismatch	2308	0.01565
3 mismatch	62	0.00085	3 mismatch	321	0.00218
4 mismatch	15	0.00021	4 mismatch	74	0.0005
5 mismatch	20	0.00027	5 mismatch	69	0.00047

chr13			chr13		
Total	102832		Total	356423	
0 mismatch	93158	0.90592	0 mismatch	308280	0.86493
1 mismatch	8672	0.08433	1 mismatch	41319	0.11593
2 mismatch	872	0.00848	2 mismatch	5614	0.01575
3 mismatch	60	0.00058	3 mismatch	831	0.00233
4 mismatch	41	0.0004	4 mismatch	234	0.00066
5 mismatch	4	0.00004	5 mismatch	54	0.00015

chr21			chr21		
Total	305545		Total	721648	
0 mismatch	268910	0.8801	0 mismatch	608536	0.84326
1 mismatch	30988	0.10142	1 mismatch	92796	0.12859
2 mismatch	3807	0.01246	2 mismatch	14506	0.0201
3 mismatch	778	0.00255	3 mismatch	3107	0.00431
4 mismatch	352	0.00115	4 mismatch	1052	0.00146
5 mismatch	373	0.00122	5 mismatch	924	0.00128

Supplementary Table 1. Deep Sequencing of Off-Target Sites. Expanded data from Figure 6. 200 bp regions of DNA with partial homology to the PNA molecule (>14 bps) in the indicated chromosomes were amplified by PCR, and PCR amplicons were sent for deep sequencing using the HiSeq (Illumina) 75 bp paired-end reads. CFBE cells treated 3 times with 2 mg/mL PLGA/PBAE/MPG particles with PNA/DNA were compared to untreated CFBE cells. The total number of aligned sequences queried, and the number of sequences with 0-5 mismatched based pairs, as well as the proportion of such sequences, are given above. Read quality data is given below:

Sequence Quality Data for Table 1:

Sequence Quality, Treated:

Read-1:

Total sequences: 2220919

Pattern trimmed: 0

Qual trimmed: 2187335

Total passed: 2187335

Short: 33584

Read-2:

Total sequences: 2220919

Pattern trimmed: 0

Qual trimmed: 2147418

Total passed: 2147418

Short: 73501

Sequence Quality, Untreated:

Read-1:

Total sequences: 4893403

Pattern trimmed: 0

Qual trimmed: 4830703

Total passed: 4830703

Short: 62700

Read-2:

Total sequences: 4893403

Pattern trimmed: 0

Qual trimmed: 4766808

Total passed: 4766808

Short: 126595

Supplementary Table 2. Primer List.

Primer Use	Sequences
CFTR gene-specific reverse primer for AS-PCR (reverse complement starting from nt 80162)	5' CCCTCTAATTCTCTGCTGGCAGATC 3'
AS-PCR forward primer for F508del	5' GCCTGGCACCATTAAAGAAAATATCATTGG3'
AS-PCR forward primer for corrected donor	5' CCTGGCACCATTAAAGGAGAAACATTATCTT 3'
AS PCR on RNA → cDNA	5' GTCTGGACGTAGACTTTGTAGCTCAG 3' 5' GCCTGGCACCATTAAAGAAAATATCATTGG3'
Regular PCR of flanking region in CFTR	5' AATGACCTAATAATGATGGGTTTATTTCCA 3' 5' ATATCTTCACAATTTTACCCCTCTAATTCT3'
Regular PCR of off-target region for sequencing	5' AAACAACTGGCCTTCATGG3' 5' GGCCACACTTTCTTCCTTCA3'
Primers for Deep Sequencing (excluding 6 base pair bar codes)	
Mouse CFTR	TCTGCTCTCAATTTTCTTGGA GGCAAGCTTTGACAACACTC
Human CFTR	TGTTCTCAGTTTTCCTGGATTATG TGGCATGCTTTGATGACG
Mouse X-chromosome donor off-target site	TGGATCTTCCTGGTGATTTTG TTATAAATTTCCCAGACTAGGCTATAA
Human chromosome 4 donor off-target site	TTCTACTAAAAGAAAACCTTCTGTGTCC CATCCCACAGACTTAATGCAAA
Primers for Deep Sequencing, Human PNA off-target sites (14 or more matched base pairs), excluding 6 base pair bar codes	
On chromosome 1:	ATTCCTGAGATAGAACAAACCATT TGAAAATGAAGCTGTTTGCTT
On chromosome 3:	TTTTCCTTCACCTTTTGAAGAA GGTGGGGCACAGTTAGAAAC
On chromosome 4:	TGTTCCATCACATAACCCCAT CCTGAATTCTGCAGCCCTTA
On chromosome 6:	CCCCCAAACACACACTGAAT AAGTTGGTTCCTTCCTCTCAA
On chromosome 8:	CATCACCTGGTGCAGGAATA TTTGCCCTTTCTGGTCATT
On chromosome 11:	TGCATATTTTCTGTCCCCTTCT AGAACTCCACCCAATGAAG
On chromosome 13:	GCCATAGCATTTTTCCTGGT TCTCACCTCCCAAACATTCC
On chromosome 14:	AAACAACTGGCCTTCATGG GGCCACACTTTCTTCCTTCA
On chromosome 15:	TCCCACCTAATAATTCCAGCA GGATGAATTATTGCCCACTGA
On chromosome 16:	GCCCACTACACCTAGGCATATC CGCTTTGTTTTCTCTCAGCA
On chromosome 17:	ACACAGCGTAAGAGGGATGG TCCACAGCATCCTAGCCTTC

On chromosome 18:	TTGTGGGAAATTTCAATGCTT TCTTGAAAGAAGCCAGAGGAA
On chromosome 21:	CCACAGATCCAGAAAGCTCAG CCTTTCTTTGTGTTTGCATGG
On chromosome X:	CCAAGCACTTTAGCCTCTGG GGAGAGGGAGAGAACAGCAA
